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ON THE MECHANISM OF SKELETAL FIXATION OF RADIUM*

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It has long been recognized that absorbed radium concentrates in the keleton. This bone-seeking property of radium has prompted a number of iorphological investigations, both of the pathological changes in the one induced by radiation[1,2,3,4] and the histological distribution of the adium itself as shown by radioautography.[5,6,7] However, there has been o comprehensive investigation of the underlying mechanisms responsible or the skeletal fixation of radium.

Recently, ionic exchange processes have been shown to be responsi- le for the skeletal fixation of a number of cations,[8] and there have been uggestions, from time to time, that radium, too, is incorporated in the iineral phase of bone by ionic exchange. With such a mechanism, the adioactive cations diffuse from the blood stream to the extracellular luids bathing the microcrystals of bone mineral. Because the crystals re so small, they present an enormous surface for an exchange[8] by which he radioactive cations displace calcium ions located in the crystals' urfaces. The present investigation was undertaken to determine whether r not ionic exchange is involved in the skeletal fixation of radium.

Dilute solutions of radium under approximately physiological condi- ions were equilibrated with a well-characterized preparation of hydroxy apatite,[9] the prototype mineral of bone.[8] The results of these studies clearly show that radium displaces calcium ions on the crystals' surfaces.

Experimental

Methods. The apparatus employed for the equilibrations is given in FIGURE 1. This is a modification of a design first described by Schweit- zer and Nehls.[10] A one-gram sample of crystals is added to 700 ml. of buffer in the flask. The suspension is stirred vigorously for 24 hours to permit the system to reach a solubility equilibrium.[11] A very small volume (0.2 ml.) of radium solution (4.6 μc.) is then added and, with stirring continuing, small aliquots of crystal-free solution are periodically withdrawn through a fine, sintered-glass filter by means of suction. Radio- activity assay of these aliquots permits the determination of the time course of the disappearance of the radium from solution or, conversely, incorporation of the radium by the crystals of hydroxy apatite.

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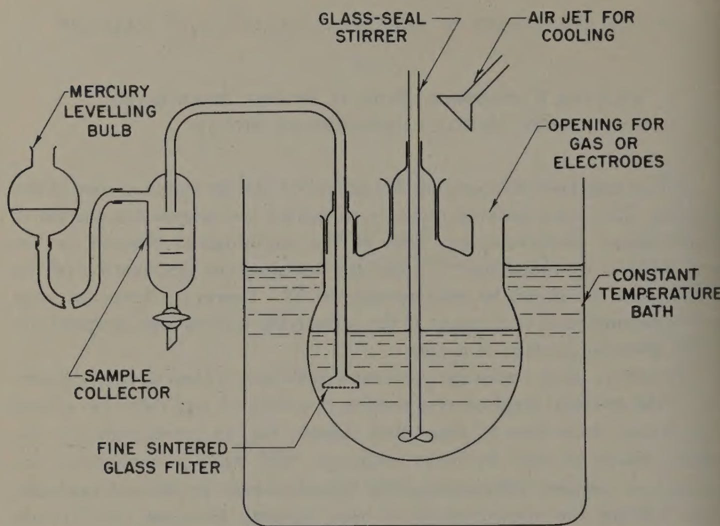

FIGURE 1. The apparatus employed for the equilibration of hydroxy apatite crystals with buffer solutions containing radium.

The solution aliquots were collected and stored in specially-constructed vials as follows: Pyrex culture tubes, 13 mm. in diameter, were sawed off at a length of 19 mm.; a 3-mm.-thick lucite disk was cemented flush to the upper lip of the shortened tube; and a glass capillary tube of 0.5-mm. bore, 15 cm. in length was cemented to a central hole in the lucite disk. The vials were checked for leaks by means of a vacuum pump. The solution sample was introduced by prior evacuation and the capillary stem then was sealed with an oxygen torch. The volume of the sample was determined by weighing on an analytical balance. After sufficient storage time for the sample to build up near-equilibrium amounts of radon, the vial was inserted into the central well of a large sodium iodide crystal, and the gamma ray activity was determined by means of a scintillation counter similar to that of Anger.[12] The counting efficiency of the method was determined by means of a known radium standard giving 1.06×10^6 net counts per minute per μc. of Ra. Sufficient counts were accumulated to keep the counting error of the order of ∓ 1 per cent. The over-all experimental error of a single determination, however, averaged ∓ 4 per cent as estimated from a statistical analysis of a large series of identical samples.

The stability of radium solutions. It was necessary first to establish

at radium solutions were stable, that radium did not adsorb on the assware or form colloids that could be filtered out under the experi-ental conditions employed.

A 1-gram batch of hydroxy apatite crystals was stirred with 700 ml. buffer: pH 7.4, potassium barbiturate 0.01 *M*, potassium chloride 15 *M*. After 24 hours, the crystals were removed by filtration through a ne, sintered filter, and the filtrate was returned to the equilibration ask. To this crystal-free filtrate, 4 μc. of radium was added and, at rying time intervals, aliquots were removed by filtration through the ntered disk (FIGURE 1).

The results, given in FIGURE 2, show clearly that, under the ex-rimental conditions employed, radium neither was adsorbed out of olution nor formed colloids capable of removal by filtration. All values greed within the $\mp$ 4 per cent over-all experimental error and agreed ith a dilution of an identical aliquot of stock radium in an $HCl{:}BaCl_2$ ixture.

The incorporation of radium by hydroxy apatite crystals. The next

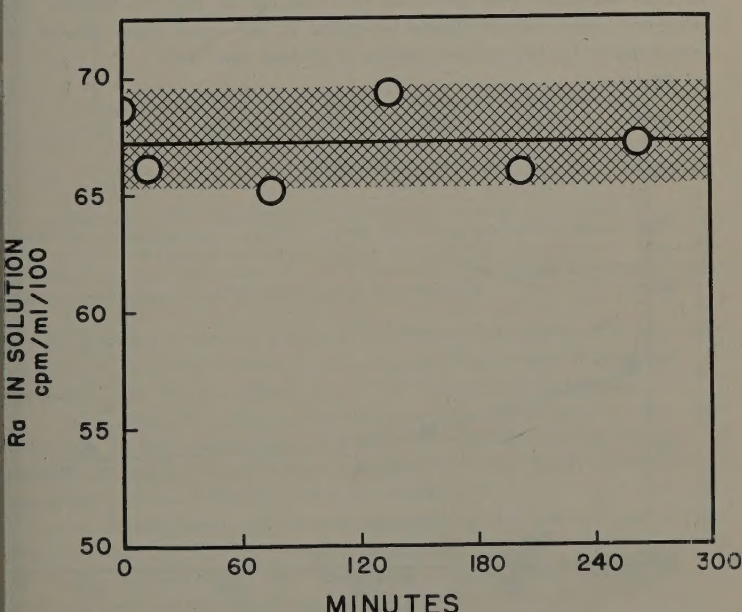

FIGURE 2. The stability of radium solutions in the absence of a mineral hase. The mean value of 6700 c.p.m. has been indicated by the horizontal line nd the grid indicates the average error.

experiment was performed in the presence of crystals. One gram of hydroxy apatite was stirred for 24 hours in 700 ml. of the buffer described above. To the crystal suspension, 4 μc. of radium was added and, as before, crystal-free aliquots were removed at intervals and assayed for radioactivity.

With crystals present, radium promptly left the solution in a reproducible fashion. The results of three separate experiments are given in FIGURE 3. The magnitude of the crystals' uptake of radium (nearly 60 per cent) is quite significant. Had the radium distributed itself uniformly on a volume basis, the crystals, *with hydration*,[13] would have removed only about 0.1 per cent of the total radium present. Clearly the mineral phase has concentrated radium either by (1) a physical adsorption on the crystal surface; or (2) an exchange of surface calcium ions for radium ions in the solution; or (3) some unknown process.

The effect of calcium ion concentration. With ionic strength (μ) kept constant, small variations in the calcium ion concentration should have but little effect on a physical adsorption. On the other hand, an exchange reaction would be very sensitive to variations in (Ca^{++}). As (Ca^{++}) is increased, less radium should be bound by the solid phase as the two ions compete for the limited number of surface positions.

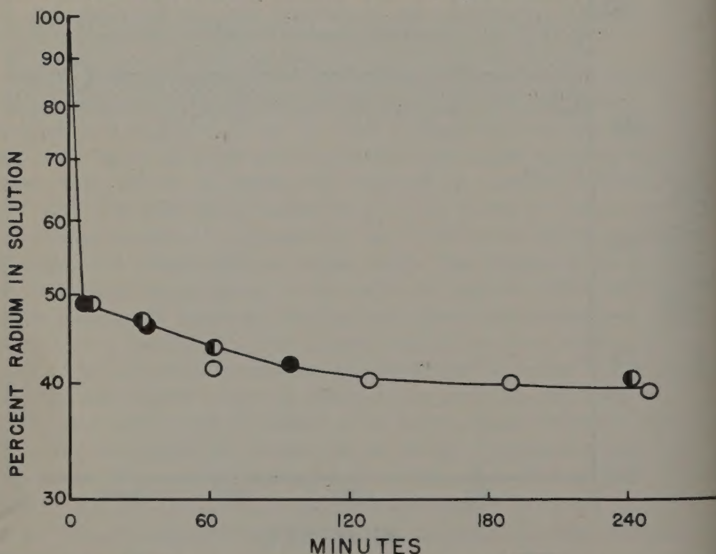

FIGURE 3. The removal of radium from solution by hydroxy apatite crystals. The results of three separate experiments are indicated by different symbols.

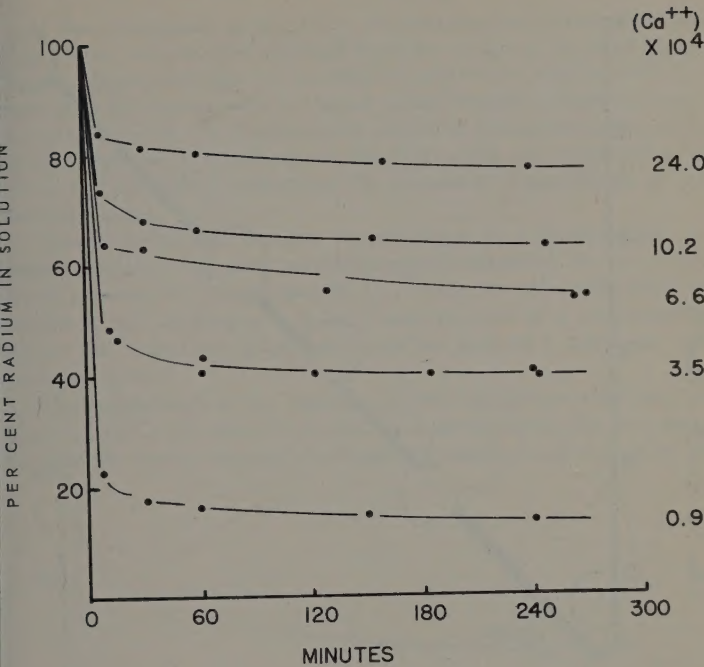

FIGURE 4. The competition between calcium ions and radium for surface positions in the hydroxy apatite crystals. In the graph, the numbers associated with each of the curves indicate the final concentration of calcium in the buffer solutions at the conclusion of the experiment. The higher concentrations of calcium prevented the removal of radium from solution.

In FIGURE 4 are the results of a series of experiments in which all variables except the calcium ion concentration were kept constant: 1 gm. crystals, 700 ml. of buffer, pH = 7.4, potassium barbiturate 0.01 *M*, μ = 0.16 (KCl). Variations in the concentration of calcium ion were achieved by small additions of $CaCl_2$ 24 hours before the addition of radium. In one instance, potassium phosphate was used to repress the amount of calcium dissolving from the crystals.[11]

The results were marked and unequivocal, the greater the concentration of calcium, the less radium incorporated by the solid phase. This is strong evidence that the underlying mechanism of radium fixation involves an ionic exchange for calcium.

An attempt was made to analyze these data quantitatively. If it is assumed that the ion-exchange process is complete for practical pur-

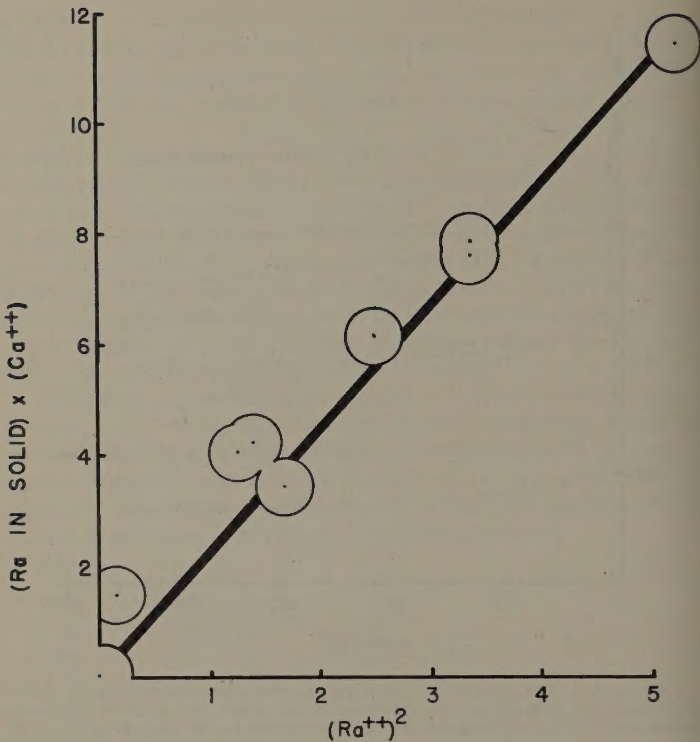

FIGURE 5. A plot of the Ra:Ca competition according to Mass Law indicating the exchange involves two moles of radium per mole of calcium. The ordinates and abscissa are expressed in arbitrary units. The diameter of the circles indicate the average error.

poses in four hours, a relation may be derived from mass law:

$$(1)\ \text{Crystal -Ca} + 2\,Ra^{++} \rightleftharpoons \text{crystal -}Ra_2^{++} + Ca^{++}$$

$$(2)\ \text{or, } (Ra^{++})^2 = k \cdot \frac{\text{crystal -}Ra_2^{++}}{\text{crystal -Ca}} \cdot (Ca^{++})$$

where "crystal $-Ra_2^{++}$ " and "crystal -Ca" are expressed as mole fractions. Since radium is present in radiochemical concentrations, the term "crystal-Ca" is essentially a constant, giving the simplified equation:

$$(Ra^{++})^2 = k \cdot k' \therefore \text{crystal } Ra_2^{++}\,(Ca^{++})$$

If then, the square of the radium concentration is plotted versus the oduct of the calcium concentration times the radium in the solid phase, linear relation *indicates* an exchange of two radium ions for each calum ion in the crystal surface. Such a linear relation is indeed obtained shown in FIGURE 5. These results should be interpreted with caution, owever, because the linearity of the curve does not constitute proof ven if the assumption concerning the attainment of equilibrium in four ours were valid.

Though surprising, perhaps, an indication of a nonequivalent exhange reaction does have well-documented precedent in the apatite ystal system: Sr^{++} exchanges for Ca^{++} mole for mole, equivalent for quivalent; Na^{+} exchanges with Ca^{++} mole for mole in a nonequivalent shion and uranyl ion displaces 2 calcium ions on a nonmolar, nonquivalent basis.[8]

The reversibility of the exchange. If the mechanism underlying the keletal fixation of radium is indeed an ionic displacement of calcium ons from the mineral crystals, the fixation process should be readily and

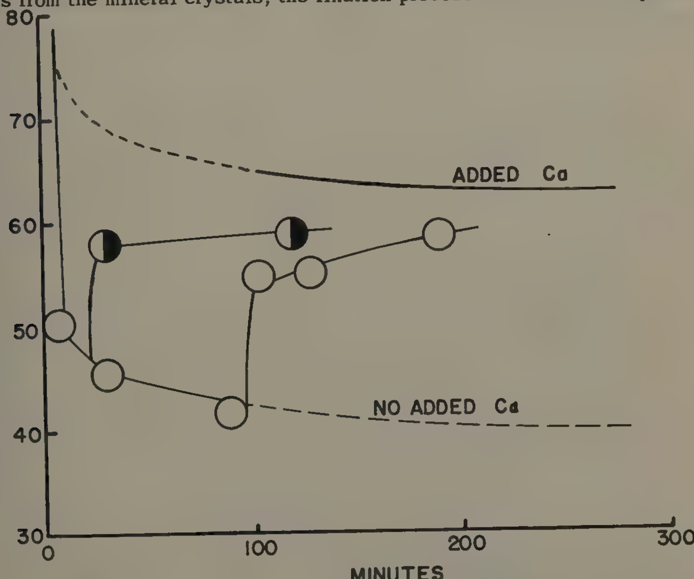

FIGURE 6. A test of the reversibility of the exchange reaction. The two curves describing the lower and upper limits represent control data from experinents in which no calcium and 35 mg. Ca/liter as calcium chloride was added, espectively, 24 hours before addition of radium. The circular symbols designate he results obtained when 35 mg. Ca/liter was added *during the course* of the exchange.

completely reversible. There are two problems, however, attendant on any attempt to demonstrate reversibility under these experimental conditions. First, the mineral phase undergoes spontaneous recrystallization,[14] which tends to "bury" newly exchanged ions and remove them from the

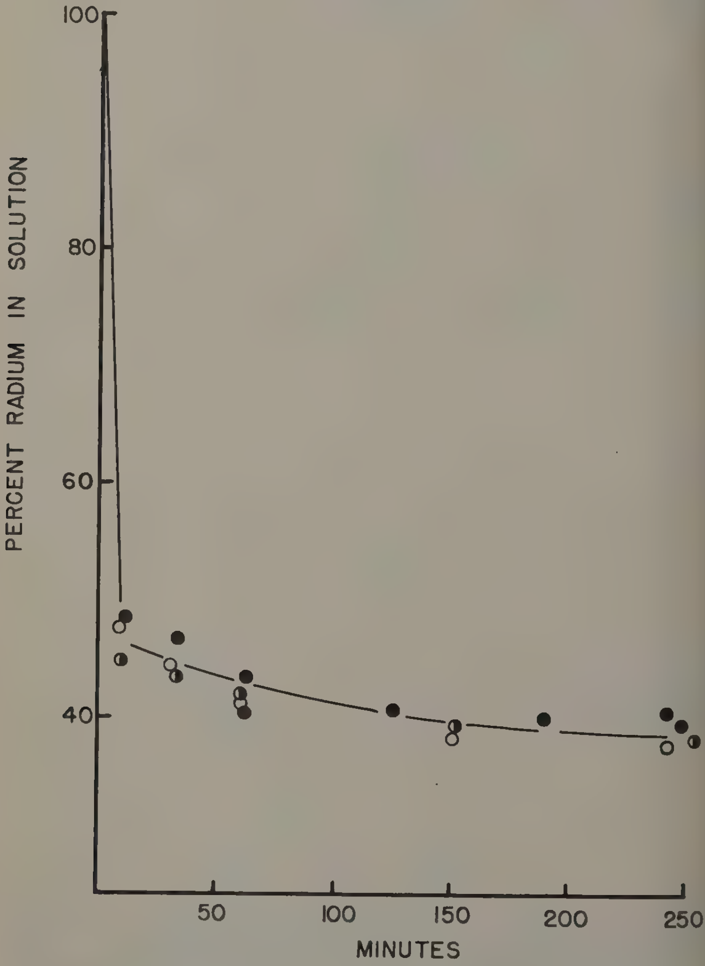

FIGURE 7. The lack of effect of sodium ion concentration on the calcium:radium exchange. Solid, open, and half solid circles represent data obtained when the buffer contained 0.00, 0.08, and 0.16 M Na, respectively.

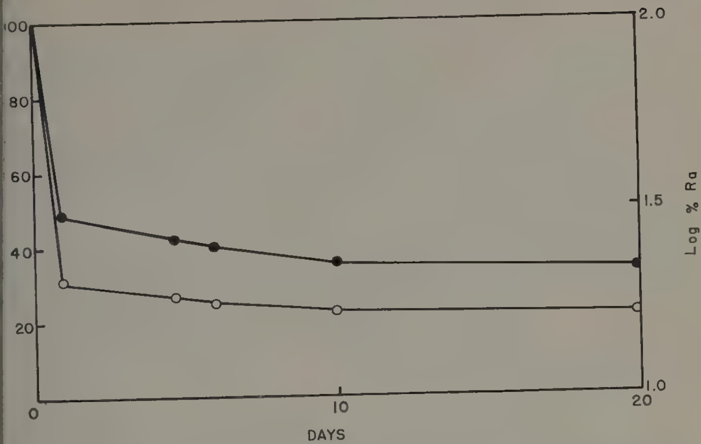

FIGURE 8. The long-term continuing removal of radium from solution by hydroxy apatite crystals. Solid circles refer to log scale; open circles refer to linear scale.

equilibrium. Second, many variables are interlocked and affect each other. For example, pH, (Ca^{++}), ($HPO_4^{=}$), solid/solution ratio, have been shown to be interrelated variables.[11]

Within the limitations of the system, then, the following experiments were conducted. A 1-gram sample of crystals was equilibrated 24 hours with 700 ml. of buffer under the usual conditions: pH 7.4, 0.01 *M* potassium barbiturate, $\mu = 0.16$ (KCl). As usual, the small aliquot of radium solution was added. After a short time interval, the course of the exchange reaction was reversed by the addition of $CaCl_2$. The results of two such experiments are given in FIGURE 6. There was a dramatically fast reversal as the added calcium displaced radium already fixed on the crystals. Within the limitations of the test itself and the experimental error of measurement, the system appeared to be reversible. The final solid:solution distribution of radium was nearly that predicted from the above "apatite uptake" curve, FIGURE 3, where all the radium was in solution at the beginning of the experiment.

The lack of influence of sodium ion. Recently, sodium ion has been shown to displace calcium ion in the surface of hydroxy apatite crystals in a fashion analogous to that proposed for radium.[8,15] The question arises as to whether sodium ion might compete with radium for the same surface positions. To answer this question, three experiments were performed in which the ionic strength of the buffer was provided by KCl, KCl:NaCl equimolar mixture and NaCl respectively. The pH, total ionic

strength, and barbital buffer concentration were all constant. The course of radium fixation was unaffected by the presence or absence of sodium ion. As shown in FIGURE 7, a single curve adequately describes all three sets of data.

This finding can be taken as evidence that sodium and radium ions do not compete for the same surface sites. This suggests that not all lattice positions of calcium are equivalent in terms of space-charge relationships.

Long-term exchange. As mentioned previously, the hydroxy apatite crystal system undergoes spontaneous recrystallization.[13] It is of prime importance to know whether radium ion can be incorporated *within* the lattice interior. If radium were limited to surfaces only, the likelihood of a successful mobilization of skeletally deposited radium *in vivo* would be much greater.

To answer this question, the exchange process was followed for protracted periods of time. Weikel has shown that a radioactively labeled ion (Ca^{45}) that can be incorporated into the lattice by recrystallization shows continuous uptake from solution by the solid phase for periods as long as 17 days.[11]

The results of the long-term experiments with radium are shown in FIGURE 8. Clearly the incorporation of radium continued for at least 10 days, possibly indefinitely. Though not unequivocal, these data support the view that radium is incorporated within the crystal interior despite the fact that the exchange for calcium ion does not appear to be on an equivalent basis, *vide supra.*

Discussion

Taken as a whole, the results of the present investigation support the following *provisional* description of the mechanisms underlying the skeletal fixation of radium. Radium ions from the extracellular fluids exchange with certain calcium ions in the surfaces of the mineral crystals of bone, and, with passing time, become incorporated to some extent within the crystals, presumably as the result of recrystallization.

Implicit in this description is the assumption that radium, in the body fluids, is freely diffusible and can enter the bone via the extracellular fluid. Fortunately, reassuring evidence on this point is available. Circulating radium has been shown to be readily ultrafiltered and to an extent greater than calcium.[16]

Though this provisional description of the fixation process requires substantiation and merits further study, it is entirely compatible with the body of physiological and morphological data thus far accumulated. For example, the exchange process predicts that the patterns of deposition

l mobilization of radium should mirror closely those of tracer doses of otopic calcium. The parallel between the two ions is, of course, well tablished.[6,7] Most important, the apparent irreversibility of the skeletal ation of radium *in vivo* is readily explained. Like Ca^{45}, radium enters, eferentially, areas of new calcification such as the growth centers of ung bones and the reforming Haversian systems in the adult. The rface-bound material gradually is incorporated within the crystals by crystallization and, as parts of this osteone, by later remodeling ocesses, become interstitial lamellae with increasing age, the incorpoted material becomes effectively isolated from the circulating fluids. is compact interstitial bone is poorly hydrated[13] and appears to be arly inert physiologically.[8]

Nothing short of heroic measures such as a nearly complete deneralization of the skeleton will be successful in the mobilization of ch isolated material. This is in keeping with the general experience those who have studied the long-term excretion of radium[17] or have nployed chelating agents in an attempt to reduce the body burden of ored radium.[18]

Summary and Conclusions

(1) Under approximately physiological conditions, dilute solutions radium were found to be stable; the radium was neither adsorbed on ass surfaces nor did it form colloidal aggregates that could be filtered.

(2) Under these conditions, however, radium was markedly removed om solution and concentrated by crystals of hydroxy apatite—the protoype mineral of bone.

(3) This fixation process was shown to involve an ionic exchange rocess in which a nonequivalent exchange of 2 moles of radium for 1 ole of surface calcium ions was indicated.

(4) In the first few hours, at least, the exchange reaction was eadily reversible.

(5) Some specificity in the lattice positions of calcium that can be ccupied by radium was observed. This was indicated by a lack of influnce by variations in sodium ion concentration.

(6) Long-term studies indicated that the incorporation of radium by he crystals continued for at least 10 days, suggesting that radium enetrates the crystal surface presumably by recrystallization of the nineral phase.

(7) It may be concluded that the ionic exchange of radium with certain calcium ions in the surfaces of the microcrystals of bone mineral s an important mechanism underlying the skeletal fixation of radium *in vivo*.

References

1. BLUM, T. 1924. J. Am. Dental Assoc. **2**: 802.
2. MARTLAND, H. S., P. CONLON & J. P. KNEF. 1925. J. Am. Med. Assoc. **85**: 1769.
3. THOMAS, H. E. & F. H. BRUNER. 1933. Am. J. Roentgenol. **29**: 641.
4. EVANS, R. D., R. S. HARRIS & J. W. M. BUNKER. 1944. Am. J. Roentgenol. **52**: 353.
5. HOECKER, F. E. & P. G. ROOFE. 1951. Radiology. **56**: 89.
6. LOONEY, W. B. & L. A. WOODRUFF. 1953. Arch. Pathol. **56**: 1.
7. ARNOLD, J. Unpublished results.
8. NEUMAN, W. F. & M. W. NEUMAN. 1953. Chem. Revs. **53**: 1.
9. NEUMAN, W. F. 1953. Univ. of Rochester Atomic Energy Rept. No. UR-238.
10. SCHWEITZER, G. K. & J. W. NEHLS. 1950. J. Phys. & Colloid Chem. **55**: 215.
11. WEIKEL, J., W. F. NEUMAN & I. FELDMAN. 1954 (June). The surface chemistry of bone. VIII. On the mechanism of ionic exchange. J. Am. Chem. Soc. In press.
12. ANGER, H. W. 1951. Rev. Sci. Instr. **22**: 112.
13. NEUMAN, W. F., T. Y. TORIBARA & B. J. MULRYAN. 1953. J. Am. Chem. Soc. **75**: 4239.
14. NEUMAN, W. F. & B. J. MULRYAN. 1952. J. Biol. Chem. **195**: 843.
15. STOLL, W. R. 1954. Federation Proc. **13**: 306.
16. PUTNAM, T. 1954. Thesis. Univ. of Rochester. Rochester, N. Y.
17. NORRIS, W. P. 1954 (June). Unpublished results reported at the 2nd Ann. Conf. on Plutonium, Radium, and Mesothorium. Univ. of Utah. Salt Lake City, Utah.
18. FOREMAN, H. 1954 (June). Unpublished results reported at the 2nd Ann. Conf. on Plutonium, Radium, and Mesothorium. Univ. of Utah. Salt Lake City, Utah.

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